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CHARACTERIZATION OF SOME INDIAN SESAME (*Sesamum indicum* L.) CULTIVARS THROUGH SOLUBLE SEED STORAGE PROTEIN MARKERS

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Divergence

Electrophoresis

Seed Coat Colour

Sesame

Protein polymorphism

Zone of adoption

ABSTRACT

Seed storage protein markers being less sensitive to environmental fluctuation than phenological traits, has been successfully employed in assessing divergence in many crop plants. The present study was aimed to find out correlation of seed storage protein markers in twenty eight Indian sesame cultivars with their agro-ecological zone of adoption and their seed coat colour. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) revealed altogether twenty two protein bands of which thirteen were polymorphic with varied molecular weights. Specific bands, relating to specific agro-ecologies were found. Moreover, bands of 93.40 KDa and 68.05 KDa were found associated with production of darker shades of seed coat colour. Clustering pattern based on protein similarity value offered no definite grouping, either to specific agro-ecological zones of adoption or to specific seed coat colour. It is concluded that individual protein banding pattern can be linked to agro-ecological adoption zone and seed coat colour which is helpful in divergence and phylogenetic study in sesame.

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1 Introduction

Sesame (*Sesamum indicum* L.) as an oilseed is potent to meet the domestic demand of edible oil in India. Though India is one of the major sesame producers in the world (www.faostat.fao.org - 2016), but this crop has been highly neglected and identified as an orphan crop. This resulted into enormous loss of germplasm and drastic reduction in variation. An insight into characterization and preservation of naturally existing variation in sesame, therefore, has become a necessity for further improvement of the crop. Phenological traits, due to pleiotropic effect and polygenic control, exhibit overlapping variation within and between species populations offering taxonomic complexity (Huber-Morath & Phlomis, 1982; Wang et al., 2010; Pabby & Rockman, 2013). Soluble seeds storage protein markers assessed through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) had been successfully employed to characterize cultivated varieties in several crop plant species, of which Mung (Ghaffor et al., 2002), Pea (Jha & Ohri, 2002), Einkorn wheat (Alvarez et al., 2006), Brassica (Khurshid & Rabbani, 2012) are few to name. Reliability of soluble seed storage protein markers lies in the fact that these are less influenced by environmental fluctuation as compared to phenological markers, therefore stable, uniform and reproducible. Moreover SDS-PAGE takes less time, is simpler to perform and is more economic than nucleic acid markers.

Only a few references are available on soluble seeds storage protein polymorphism study involving Indian sesame germplasm. Akhila & Beevy (2011) reported the presence of fourteen bands in a study of seven sesame genotypes including wild and cultivated species. The protein polymorphism was able to group the genotypes belonging to two different species into separate clusters. Das et al. (2013) reported the presence of twenty two bands in a study with twenty six advanced sesame mutant lines and respective controls. But the study did not offer much specificity in grouping the mutants according to their parental origin. Dar et al., (2014) reported as much as twenty one bands in a study with fifty two Indian sesame germplasms. Similarly, Singharaj & Onsaard (2015) carried out SDS-PAGE in sesame genotypes with varied seed coat colour, for its food value. According to recent study, seed coat colour in sesame is more helpful in phylogenetic study than geographic origin of genotypes (Zhang et al., 2013).

In the present research work an attempt was made to search for correlation between soluble seed storage protein polymorphism of a number of Indian sesame cultivars with the cultivars' agro-ecological zone of adoption and also with cultivars' seed coat colour, which would definitely incite more knowledge on divergence and phylogeny in sesame. Correlation of protein markers with seed coat colours would further help in assessing genotypes for those biochemical traits which are linked to particular seed coat colours (Zhang et al., 2013). Such correlation, if exists, can be employed to identify diverse and superior sesame genotypes for future crop improvement programme.

2 Materials and methods

Twenty eight sesame cultivars from different states in India representing varied agro-ecology had been selected for the study. The detail of the genotypes is presented in Table 1. Seeds of these cultivars were availed from the sesame germplasm collection of the Department of Genetics and Plant Breeding,

Table 1 Detail of cultivars under study

Genotypes	Seed coat colour	State: Agro-ecological zone of adoption*
AMRIT, NIRMALA, UMA	Pale yellow	Odisha: Sub-humid, Coastal
OSC-207, OSC-593	White	
TKG-352, TKG-22	White	Madhya Pradesh: Semi-arid, sub-humid
DSS-09	White	Karnataka: Arid, Semi-arid
TMV-4, TMV-6, VRI-1	Brown	Tamil Nadu: Semi-arid, Coastal
GUJARAT TIL-2 (GT-2)	White	Gujarat: Arid, Semi-arid, Coastal
CST-2001-12	White	Haryana, Rajasthan: Arid
RT-54, RT-348	Brown	
B-14, TILOTTAMA (B-67), CUMS 3, V-1, V-15, RAMA	Brown	West Bengal: Humid- per humid, Coastal
CUMS 9, CUMS 11	Mixed colour [1]	
CUMS 17, NIC 8316, SAHEB	Pale yellow	
V -10, V-12	Deep Brown	

*Source: ICAR-Indian Agricultural Statistics Research Institute; [1] Mixed colours seeds included pale yellow, brown, deep brown

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Soluble seed storage protein was extracted and estimated following Lowry's method (Lowry et al., 1953). SDS-PAGE (12% separating gel and 4% stacking gel) of those extracted protein was carried out following the method of Laemmli (1970) in a regular mini (10 cm x 10 cm) vertical gel system (Biotech Laboratories, India) applying a current of 30 mA for run through stacking gel and a current of 50 mA for run through separating gel. Standard marker protein, Dalton Mark VI (Sigma, USA) was used as a control. Staining and de-staining of the gel were also carried out following the method of Laemmli (1970). The banding pattern was captured through gel documentation unit (UVP Gel Doc It) and molecular weights (MW) of observed protein bands were then estimated through the Life Sciences Software available with the gel documentation unit. A matrix was prepared by giving a score of '1' for presence and '0' for absence of a particular protein band. This was carried out for all the observed protein bands for all twenty eight genotypes. A dendrogram was prepared based on protein similarity (PS) value of the genotypes with the help of the software NTSYS pc ver 2.20 (Rohlf, 2005). Protein similarity data were generated by the program, SIMQUAL and the dendrogram was generated through SAHN program. Protein dissimilarity (PD) was estimated as 1 - PS.

3 Results

The SDS-PAGE banding pattern for soluble seed storage protein revealed a total of twenty two bands covering all the twenty eight genotypes as reported earlier by the authors (Das et al., 2013) in their previous study on seed storage protein polymorphism involving sesame mutants and their parents. The heaviest band at the top of the gel with molecular weight of 125.59 KDa was marked as one (1), the lightest polypeptide was the 22nd band of 12.19 KDa found at the bottom. All the other polypeptides found in between these two bands had molecular weights ranging in between 125.59 KDa and 12.19 KDa. Sigma, Dalton Mark VI gave six marker bands of molecular weights 66 KDa, 45 KDa, 34.7 KDa, 24 KDa, 18.4 KDa and 14.4 KDa respectively, distributed from top to bottom of the gel. Marker band of 45 KDa divided all the bands almost at the mid-way. Ten bands appeared above 45 KDa band and twelve below. Besides, variation in total number of bands was observed in different genotypes offering seed storage protein polymorphism of 59.1%. A zymogram for ten genotypes is given in Figure 1, along with marker bands. Seven protein bands, namely, band no. 6, 7, 9, 10, 14, 15, 18, 20 and 22, out of these twenty two bands were monomorphic. The other thirteen bands, namely, band no. 1, 2, 3, 4, 5, 8, 11, 12, 13, 16, 17, 19 and 21 exhibited polymorphism. The detail of the polymorphic bands is given in Table 2.

Table 2 Detail of polymorphic bands

Band Position	Molecular weight (KDa)	Band Position	Molecular weight (KDa)	Band Position	Molecular weight (KDa)
1	125.59	8	68.05	17	22.37
2	116.32	11	45.46	19	18.14
3	107.20	12	42.25	21	14.07
4	102.39	13	33.23		
5	93.40	16	24.96		

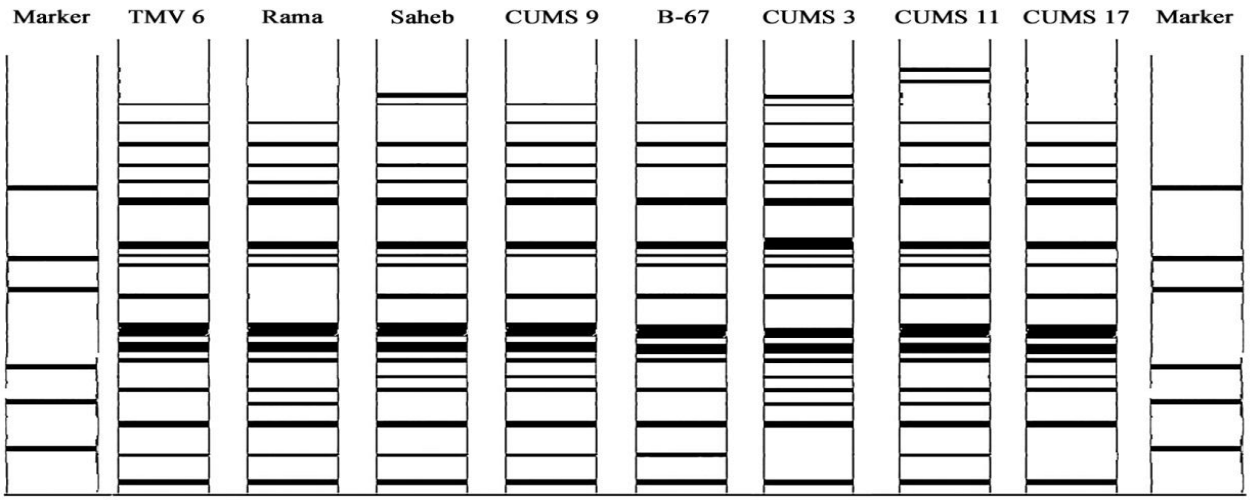

Figure 1 Zymogram for SDS-PAGE banding pattern of ten sesame cultivars with markers

3.1 Protein banding pattern for agro-ecological zone of adoption

The distribution of polymorphic protein bands in all the cultivars is presented in Table 3. The distribution pattern revealed the presence or absence of some specific polypeptides or protein bands which were associated with specific agro-ecology as explained here. It is evident from Table 3 that polypeptides of 68.05 KDa, 42.25 KDa, 33.23 KDa & 24.96 KDa were present in all the cultivars except those from Haryana, Rajasthan and West Bengal. Absence of these bands can be related to the specific agro-ecology of these three states which was very different from all the other states (Table 1). While humid coastal agro-ecology of West Bengal might be associated with the unique presence of 45.46 KDa polypeptide in all the cultivars from this state, the arid agro-ecology of Haryana and Rajasthan was convincingly linked with the unique presence of protein bands of 116.32 KDa and 107.20 KDa in the representative cultivars. The banding pattern also revealed that, polypeptide of 93.40 KDa (Band no. 5) can be considered as the linking polypeptide between arid and semi-arid agro-ecology, while that of 14.07 KDa (Band no. 21) must be the link between semi-arid, sub-humid & coastal agro-ecology. Presence of protein band of 68.05 KDa (Band no. 8) was a typical to the cultivars from those states which covered a range of agro-ecological zones rather than a single one.

3. 2. Protein banding pattern for seed coat colour

In case of seed coat colours, unique protein banding pattern was revealed as represented in Table 4. Protein bands of 42.25 KDa, 33.23 KDa & 24.96 KDa (Band no. 12, 13, 16 respectively) was present uniformly in the white seeded cultivars, while presence of polypeptides of 68.05 KDa & 45.46 KDa (Band no. 8 & 11 respectively) additional to band no. 12, 13, 16 was found for pale yellow seed coat. Polypeptide of 93.40 KDa was most probably related to darker seed coats, namely, brown and deep brown (Band no. 5). All the protein bands except that of 68.05 KDa, specific to different seed coat colours were present in the cultivars with mixed seed coat colours, ranging from pale yellow to brown to deep brown, which confirms the association specific polypeptides to specific seed coats as discussed. Absence of 68.05 KDa protein must had been compensated by the specific presence of polypeptide of 18.14 KDa in these mixed coloured cultivars only. It can be concluded from Table 4 that brown and dark brown seed coat were probably the results of complex interplay of reaction due to presence and absence of protein bands of 68.05 KDa and 24.96 KDa along with other three bands of 42.25 KDa, 33.23 KDa & 24.96 KDa.

Table 3 Distribution of protein polymorphic bands for agro-ecological zone of adoption

Agro-ecological zone of adoption	State	Band position[1]
Sub-humid, Coastal	Odisha	8, 12, 13, 16, 21
Semi-arid, sub-humid	Madhya Pradesh	5, 8, 11, 12, 13, 16, 21
Arid, Semi-arid	Karnataka	8, 12, 13, 16, 21
Semi-arid, Coastal	Tamil Nadu	4, 8, 12, 13, 16, 21
Arid, Semi-arid, Coastal	Gujarat	5, 8, 11, 12, 13, 16
Arid	Haryana, Rajasthan	2, 3, 5, 12, 3, 16
Humid-per humid, Coastal	West Bengal	11

[1]Positions of those polymorphic bands which were uniformly present in all genotypes specific to a particular zone of adoption and seed coat colour. The molecular weights of the bands can be found from Table 2.

Table 4 Distribution of protein polymorphic bands for seed coat colour

Seed coat colour	Band position[1]
White	12, 13, 16
Pale yellow	8, 11, 12, 13, 16
Brown	5, 16
Dark brown	5, 8, 11, 12, 13
Mixed	5, 11, 12, 13, 16, 19

[1]Positions of those polymorphic bands which were uniformly present in all genotypes specific to a particular seed coat colour. The molecular weights of the bands can be found from Table 2.

3. 3. Dendrogram and clustering pattern

The dendrogram (Figure 2) computed from the polymorphic banding pattern, revealed complete linkage between genotypes at specific distances. It revealed two clusters, as marked by the parentheses. The cluster with larger number of cultivars was divided into five sub-clusters marked by straight lines. Genotypes within same cluster shared high degree of similarity with each other than genotypes in different clusters. Highest similarity coefficient of 1.0 was observed between TKG-22 and TMV-6, belonging to two distinctly different agro-ecological zones (Table 1) and least similarity (0.73) was observed between OSC -207 and CUMS 17 also from two states with very different agro-ecology (Table 1). The clustering pattern revealed no specific correlation of protein similarity of the cultivars, neither to the cultivars' zone of adoption, as reported earlier by Dar et al. (2014), nor with their seed coat colour. This outcome is of common occurrence with nucleic acids markers also (Pham et al., 2009; Wei et al., 2008).

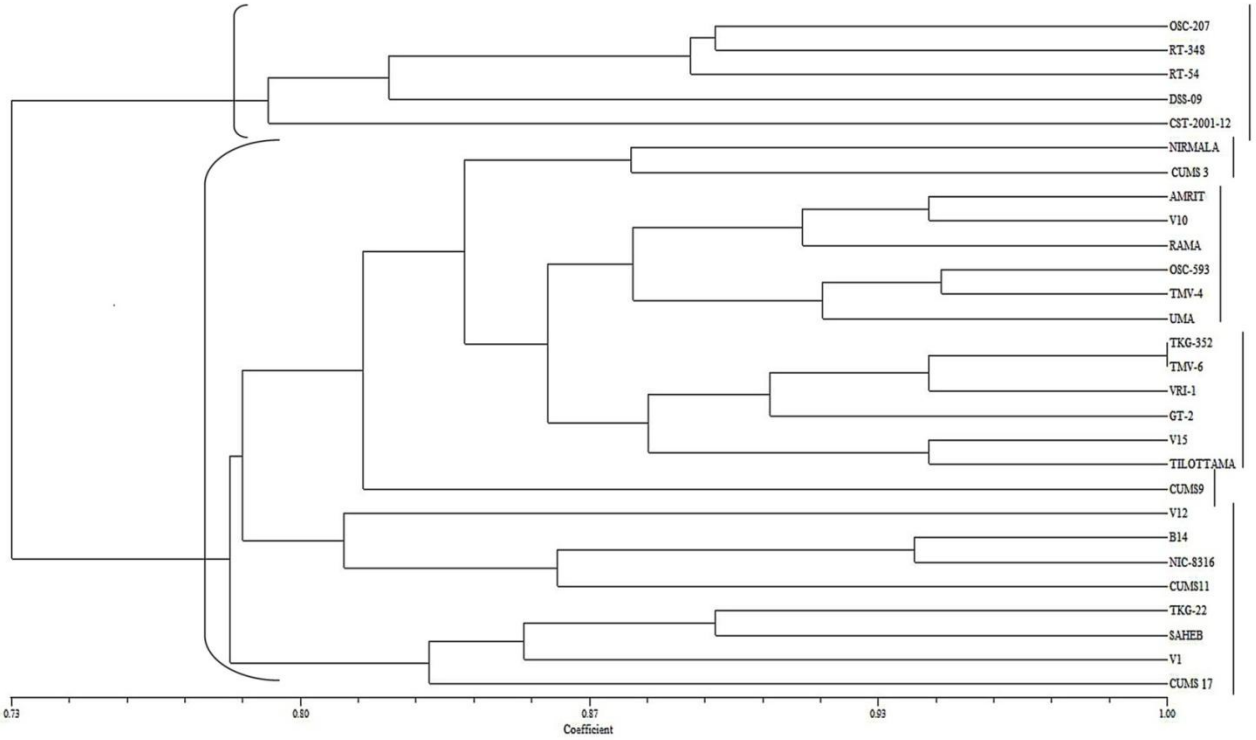

Figure 2 Dendrogram of twenty eight sesame cultivars based on soluble seed storage protein polymorphism

4 Discussion and conclusions

Human interference played a great role in spreading crop cultivars to different regions, which probably led to lack of correlation in clustering of cultivars with zone of adoption (Stankiewicz et al., 2001). Moreover, it is well known that a single agro-ecology can be sub-divided into a number of agro-climatic zones and sub-agro climatic zones which leads to a range of genotype and environmental interaction (GE) within a single agro-ecology. In India, fifteen agro-ecological regions have been sub-divided into as many as 127 agro-climatic regions (vikaspedia.in). In the present study, similar to findings of Akbar et al. (2012), specific polypeptides were obtained related to specific agro-ecology, like that of West Bengal (humid), Haryana (arid) and Rajasthan (arid), but it was not possible to identify unique protein bands specific to those cultivars which were originated in the states with mixed agro-ecology.

Hence to draw any definite inference on divergence, phylogeny and pattern of distribution of sesame populations in Indian sub-continental context, more genotypic representation from different agro-climatic regions are required. Cultivar specific polypeptides and identifying specific protein fractions is also a potent field for a more precise outcome. Seed coat colour in sesame is linked to other important biochemical traits (Kanu, 2011; Zhang et al., 2013). Assessment of such traits, like, oil content, antioxidant content, free fatty acid content & many others, and linking such traits to seed coat colour specific - polypeptides will definitely help in screening superior and diverse sesame genotypes for crop improvement programs more precisely.

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